

APPENDIX D

WATER-EFFECT RATIO DETERMINATION FOR HEXAVALENT CHROMIUM

SUMMARY

The WER procedure was selected to develop a site-specific CMC for hexavalent chromium for the effluent's receiving stream, Little Hollow Run. WER procedures followed those described in USEPA's "Interim Guidance on Determination and Use of Water-Effect Ratios for Metals" (1994). The *Daphnia magna* 48-hour static acute toxicity test was selected as the primary toxicity test for use in three seasonal WER determinations (sample dates: May 9, July 17, and September 11, 1995). The fathead minnow 48-hour static acute toxicity test was selected as the secondary toxicity test for use in one of the WER determinations (sample date: July 17, 1995). Site water used in all three WER determinations was undiluted effluent since the receiving stream originates at the discharge point of the outfall. Soft Reconstituted Water (SRW) and Moderately Hard Reconstituted Water (MHW) were used as the laboratory water for the primary and secondary toxicity tests, respectively. To assess the possible effects of hardness and pH, a MHW test was conducted with each primary toxicity test WER determination.

The primary toxicity test WERs determined from the samples collected on May 9 (spring), July 17 (summer) and September 11 (fall), 1995 were 7.92, > 24.15, and 7.29, respectively. The reason for the higher WER for the summer WER determination was due to the combined effect of a lower SRW LC₅₀ and a higher site water LC₅₀. The lower summer SRW LC₅₀ was apparently due to natural test variation, whereas the higher summer site water LC₅₀ may have been due to a change in the character of the site water such as higher pH (summer site water pH, 8.1; spring and fall site water pH, 7.5). The secondary toxicity test WER determination was considerably lower (0.88) than the primary toxicity test WERs. The lower WER for the secondary toxicity test was a validation of the primary toxicity test WERs in that less sensitive toxicity tests are expected to produce a lower WER than a more sensitive toxicity test (*D. magna* is approximately 1000X more sensitive to hexavalent chromium than fathead minnows). The final WER for hexavalent chromium determined from this study is 7.29 (lowest of the three primary toxicity test WERs) and the corresponding site-specific CMC for Little Hollow Run is 109.4 µg/L (15 µg/L hexavalent chromium x 7.29).

METHODS

Water-Effect Ratio

Site Water

Site water consisted solely of undiluted effluent collected from the power plant's outfall. 24-hour composite samples of the effluent discharge were collected on May 9, July 17 and September 11, 1995 for the three different WER determinations. The samples were collected in polyethylene cubitainers, which upon collection were placed in a cooler containing wet ice. The samples were transported to the testing laboratory on the day the samples were collected. Upon receipt, the samples were logged in, given an identification number, measured for temperature, pH, dissolved oxygen, specific conductivity, hardness and alkalinity, filtered through a 95 µm nylon screen (to remove unwanted organisms), and placed in a 4EC refrigerator. Copies of the chain-of-custody form for the effluent sample (including samples submitted for chemical analysis) and the effluent characterization form containing the initial water quality measurements are presented in Appendix A (not included in this case study).

Laboratory Water

SRW and MHW were used as dilution water for the laboratory water tests. SRW and MHW were prepared using a Standard Operating Procedure (SOP) which is based on instructions cited in Weber et al. (1991). Base water used in the preparation of the reconstituted waters was deionized water from a Millipore Milli-Q™ Plus water system. Reagent grade salts were added in the appropriate amounts to deionized water and mixed at room temperature.

A summary of the water quality characteristics of the effluent sample and the two reconstituted waters for the fall sample date are given in Table 1.

Stock and Test Solution Preparation

Reagent grade hexavalent chromium obtained from Aldrich Chemical Company, Inc. on December 1, 1994 was used to make the stock and test solutions in all three WER determinations. The day before the WER tests were initiated, a 250 mg/L stock solution was prepared by dissolving 70.7 mg hexavalent chromium in 100 ml Millipore (deionized) water. Test solutions (the solution to which test organisms were exposed) were prepared the day the WER tests were initiated in the following manner:

- An appropriate volume of stock solution was added to a measured volume of dilution water and mixed.
- The spiked test solution was allowed to equilibrate for three hours.
- The spiked test solution was serially diluted with unspiked dilution water using a 0.7X dilution factor.
- The diluted test solutions were allowed to equilibrate approximately one hour before initiating the tests.

A detailed description of the stock and test solution preparation is given in the laboratory information sheet in Appendix C (not included in this case study).

WER Toxicity Test Procedures

All procedures followed the study-specific SOP for conducting a 48-hr *D. magna* static acute toxicity test as presented in the Study Plan for this project. A summary of the test conditions were as follows:

| | |
|------------------------|--|
| Test Chamber | 250 ml Glass Beaker |
| Depth of Solution | 40 mm |
| Volume of Solution | 125 ml |
| # of Organisms/Chamber | 5 |
| Lighting | 16:8 light dark photoperiod; 10-20 $\mu\text{E}/\text{m}^2/\text{s}$ |
| Test Initiation | Date and Time |
| Test Termination | Date and Time |

In an attempt to eliminate the occurrence of "floating" organisms, 200 μm nitex screens were inserted into each *D. magna* test beaker using a coiled polyethylene ring to maintain the screen immediately below the water's surface.

Test Organisms

Stock cultures of *D. magna* used in the WER were originally obtained from Aquatic BioSystems, Inc. in November 1994 (see Organism History, Appendix D – not included in this case study). *D. magna* were cultured at the testing laboratory in MHW and a natural surface water in environmental chambers under controlled conditions (temperature $20\text{E} \pm 2\text{EC}$; photoperiod 16 h light and 8 h dark). *D. magna* were cultured in 1 L glass beakers containing approximately 800 ml of MHW or 0.45 μm filtered Boardman river water. Daily, each beaker received 5 ml of a yeast/trout food/Cerophyl® (YTC) food suspension (see Weber et al., 1991; EPA/600/4-90/027 for procedures for preparing the food suspension) and 0.8 ml of 2.3×10^8 cells/ml of the green alga, *Selenastrum capricornutum*. Culture water in each beaker was changed a minimum of three times each week. On the days the culture water was not changed, all young were removed from each culture beaker. Survival and reproduction of culture animals and general water chemistry was measured (dissolved oxygen, pH, temperature, and specific conductivity) and recorded each time culture water was changed. Test animals were obtained from cultures where survival of culture animals was 100 percent and reproduction was ≥ 3.0 young per female per reproductive day. Twenty-four hours before the start of a test, all young were removed from the culture chambers to ensure that only daphnids less than 24-hours old would be available to start the test. Copies of the culture records for the acclimation cultures used in the fall WER are given in Appendix D (not included in this case study).

Ten organisms randomly selected from the MHW control beakers at the end of the fall sample test were measured using a Wild dissecting microscope equipped with an ocular micrometer. The average length (tip of head to base of spine) of the ten daphnids was 1.08 mm (range, 0.96 to 1.20 mm). Verification that the test organisms used in all WER tests were *D. magna* was made by processing representative individuals from the *D. magna* culture through the dichotomous key in Edmondson (1959).

Measurements

General water quality conditions and hexavalent chromium were measured in the effluent samples and in the test solutions of the 48-hour WER toxicity tests. The type of chemical measurements made in the effluent samples and toxicity test solutions and the method used to perform the measurement are presented in Table 2.

RESULTS

Water-Effect Ratio Tests

Primary Toxicity Test

The average and range of pH, dissolved oxygen, temperature, and specific conductivity in the test solutions of the primary WER toxicity tests are given in Table 3. The hexavalent chromium and *D. magna* 48-hour survival measurements for the site water, SRW and MHW toxicity tests are given in Tables 4, 5, and 6, respectively. The hexavalent chromium concentrations measured at test initiation (Day 0) and at test termination (Day 2) were not appreciably different from each other and were similar to the target nominal concentrations. The Spearman-Kärber method was used to calculate LC₅₀ values for each test using both the nominal and the measured hexavalent chromium concentrations. The average of the hexavalent chromium concentrations measured at Day 0 and Day 2 were used for the measured concentrations in the LC₅₀ calculation. The LC₅₀ values for the site water, SRW and MHW tests using the average measured hexavalent chromium concentrations were 173.37, 23.77, and 49.06 µg/L, respectively. The LC₅₀ value for the SRW test (23.77 µg/L) was very similar to the two hexavalent chromium LC₅₀ values for *D. magna* (24.2 and 22 µg/L) reported in the ambient Water Quality Criteria Document for hexavalent chromium (EPA 440/5-84-999) which were conducted at a similar hardness (45 mg/L as CaCO₃). Copies of the data sheets containing the individual physical, chemical and survival measurements, and printouts of the statistical analyses are given in Appendix E (not included in this case study).

Calculation of Water-Effect Ratio

A WER of 7.29 was calculated for the fall sample using the measured LC₅₀ values for the site water and SRW tests (Table 4).

DETERMINATION OF THE FWER

Summary of Three WER Determinations

The LC₅₀ values for the primary and secondary species for each water type and the respective WERs for the spring, summer, and fall samples are summarized on Table 7. The results of the May 9, 1995 (spring) and September 11, 1995 (fall) samples were very similar. The two samples had very similar LC₅₀ values for all three water types and therefore had very similar WERs (spring, 7.92; fall, 7.29). The results of the July 17, 1995 (summer) sample were somewhat different than the spring and fall samples in that the summer sample WER of > 24.15 was considerably higher than the other two WERs. The relatively high summer WER was due to the SRW LC₅₀ for the summer WER being lower than the spring and fall WER determinations and the site water LC₅₀ being higher than the spring and fall WER determinations (Table 7). The reason for the lower LC₅₀ for the summer SRW test seems to be attributable to natural test variation. Factors such as unhealthy test organisms, differing water quality characteristics, and an error in

the dosing of hexavalent chromium do not appear to be the cause of the lower SRW LC₅₀ value for the following respective reasons:

- 1) the LC₅₀ value for the summer MHW test was very similar to the spring and fall tests, and the reference toxicant LC₅₀ value for July was within specifications,
- 2) the alkalinity, hardness, conductivity, and pH of the summer SRW was very similar to the spring and fall SRWs, and
- 3) the measured hexavalent chromium values are very similar to the target concentrations.

The reason the site water LC₅₀ value for the summer sample is higher than the spring and fall samples may be due to natural test variation and/or differences in the water quality characteristics of the effluent samples. The measured water quality parameters represent only a portion of all constituents in the treated fly ash effluent; other unmeasured parameters may have contributed to the differential toxicity response between seasonal tests. The water quality characteristics of the three samples (fall sample, Table 1; spring and summer samples, Appendix F – not included in this case study) are different, but the only parameter that sets the summer sample apart from both the spring and fall sample is pH which is higher in the summer sample (summer sample pH, 8.1; spring sample pH, 7.5; fall sample pH, 7.5). Call *et al.* (1981) found that hexavalent chromium was less toxic to *D. magna* in water with a higher pH which is similar to that observed in the summer test. A review of the mortality data for the three tests reveals a very similar concentration-response through the 210 µg/L nominal test concentration for all three tests, but a change in the 300 µg/L nominal test concentration was noted, in that the summer test had 55 percent survival and the other two tests had 0 percent survival. In summation, the difference in the results of the summer site water test may be due to pH, a water quality characteristic not measured or a combination of water quality characteristics, or, as in the SRW test, natural test variation.

Quality Assurance Criteria

Acceptability of Laboratory Dilution Water

As stated in the WER guidance document (EPA-823-B-94-001), two sensitive tests using the laboratory dilution water must be compared to the results obtained in another laboratory using similar water. The following table presents the three sensitive tests conducted with *D. magna* in SRW during this study and the results of two different tests obtained from the "Ambient Water Quality Criteria for Hexavalent Chromium" document (USEPA, 1985) which used a similar dilution water. Two tests, spring and fall, are within a factor 1.5X of the comparison tests which fall within the recommended criterion for lab water acceptability.

| Test Reference | LC ₅₀ , µg/L | Hardness, mg/L as CaCO ₃ | Alkalinity, mg/L as CaCO ₃ | pH, S.U. |
|-------------------------|----------------------------|--|--|-------------|
| SRW - Spring Sample | 24.84 | 44 | 34 | 7.8 |
| SRW - Summer Sample | < 13.5 | 40 | 32 | 7.8 |
| SRW - Fall Sample | 23.77 | 40 | 28 | 7.6 |
| Mount, 1982 | 24.2 | 45 | not known | not known |
| Mount and Norberg, 1984 | 22 | 45 | 43-45 | 7.2-7.4 |

Secondary Toxicity Test

A fathead minnow 48-hour static acute toxicity test was conducted on MHW and site water collected July 17, 1995 (summer sample). This test represented the required secondary toxicity test. The LC₅₀ values for hexavalent chromium in the MHW and site water were 78.71 and 69.36 mg/L, respectively, which are slightly higher than the 31 LC₅₀ values (mean = 41.6 mg/L; range, 17.6-66 mg/L) reported for fathead minnows in the "Ambient Water Quality Criteria for Hexavalent Chromium" document (USEPA, 1985). The WER for the secondary test (0.88) was considerably lower than the *D. magna* WERs. As stated in the WER guidance, a less sensitive test will probably give a smaller WER than a more sensitive test. The reason the more sensitive tests will result in a higher WER can be explained using a simplified example, in which the site water contains a complexing agent which renders the hexavalent chromium nontoxic. EXAMPLE: The concentration of a complexing agent in the site water is 500 µg/L and has the ability to bind 100 µg/L of the hexavalent chromium. For the *D. magna* toxicity test which has an endpoint in laboratory water of approximately 20 µg/L, it will require at least 120 µg/L of metal in the site water (100 µg/L will be bound to complexing agent) to produce the endpoint concentration of 20 µg/L, resulting in a WER of approximately 6. For the fathead minnow 48-hour acute toxicity test, which has an endpoint in laboratory water of approximately 60,000 µg/L, it will require at least 60,100 µg/L of hexavalent chromium in the site water (100 µg/L bound to the complexing agent) to produce the endpoint concentration of 60,000 µg/L, resulting in a WER of approximately 1. The difference in the WERs between the primary and secondary tests in the present study is a validation of the *D. magna* WERs because a low to negligible WER was anticipated for fathead minnows given their relative tolerance to hexavalent chromium (approximately 1000 times less sensitive than *D. magna*).

Final Water-Effect Ratio

The site water for all three WER determinations was undiluted effluent (no upstream receiving water), and therefore the WERs determined for each sample are the same as highest WER (hWER). The USEPA guidance states that when two or more Type 1 WERs are determined (total number in this study is three), and when less than nineteen percent of all WERs are Type 2 WERs (total number of Type 2 WERs in this study is zero), the FWER is the lowest Type 1 WER or the lowest hWER. Based on this guidance the FWER for this site determined from this study is 7.29. Thus, the corresponding site-specific water quality criterion modification for Little Hollow Run would be $15 \mu\text{g/L Cr } 6^{+} \times 7.29 = 109.4 \mu\text{g/L}$.

KEY PERSONNEL

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Project Quality Assurance Officer for WER Study: Greg Smith, GLEC

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TABLE 1. WATER QUALITY CHARACTERISTICS OF RECONSTITUTED AND SITE WATER USED IN THE FALL WER DETERMINATION

| Water Quality Characteristic | Composite Effluent Sample | SRW Batch No. 9 | MHW Batch No. 82 |
|---------------------------------------|---------------------------|-----------------|------------------|
| pH, S.U. | 7.5 | 7.6 | 7.9 |
| Dissolved Oxygen, mg/L | 9.5 | 8.6 | 8.7 |
| Specific Conductivity, μ mhos/cm | 1072 | 146 | 271 |
| Alkalinity, mg/L as CaCO ₃ | 28 | 28 | 60 |
| Hardness, mg/L as CaCO ₃ | 416 | 40 | 84 |
| TOC, mg/L | 1 | not meas. | not meas. |
| TDS, mg/L | 884 | not meas. | not meas. |
| Hexavalent chromium, μ g/L | 4.5 | < 3 | < 3 |

TABLE 2. SAMPLING SCHEDULE AND METHOD FOR CHEMICAL MEASUREMENTS

| Parameter | Sample Type(s) | Sampling Schedule | Method |
|----------------------|--------------------|--------------------------------------|-------------------------------|
| pH | Effluent Composite | Sample Receipt | Electrometric, EPA 150.1 |
| | WER Tests | Test initiation and test termination | |
| Specific Conductance | Effluent Composite | Sample Receipt | EPA 120.1 |
| | WER Tests | Test initiation | |
| Dissolved Oxygen | Effluent Composite | Test initiation | Membrane Electrode, EPA 360.1 |
| | WER Tests | Test initiation and test termination | |
| Hardness | Effluent Composite | Sample Receipt | Titrimetric, EPA 130.2 |
| Alkalinity | Effluent Composite | Sample Receipt | Titrimetric pH 4.5, EPA 310.2 |
| Hexavalent chromium | Effluent Composite | Test initiation | EPA 218.4 |
| | WER Tests | Test initiation and test termination | |
| TOC | Effluent Composite | Test initiation | EPA 415.1 |
| TDS | Effluent Composite | Test initiation | EPA 160.1 |

TABLE 3. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN *Daphnia magna* TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED SEPTEMBER 12-14, 1995

| Parameter | Site Water Test | | SRW Test | | MHW Test | |
|--|-----------------|---------------------|-------------|---------------------|--------------|---------------------|
| | Average | Range | Average | Range | Average | Range |
| PH, S.U. | 7.6 | 7.5-7.9 | 7.6 | 7.5-7.8 | 8.0 | 7.8-8.1 |
| Dissolved Oxygen, mg/L (% Saturation) | 8.9 (101) | 8.2-9.5 (93-108) | 8.6 (98) | 8.5-8.9 (97-101) | 8.8 (100) | 8.5-9.0 (97-102) |
| Temperature, EC | 19.8 | 19.4-20.1 | 19.9 | 19.4-20.1 | 19.9 | 19.7-20.1 |
| Specific Conductivity, μ mhos/cm | 1102 | 1072-1123 | 145 | 144-146 | 276 | 255-286 |

TABLE 4. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO SITE WATER (UNDILUTED EFFLUENT)

| Results of WER Determination Test Dates: September 12 - 14, 1995 SITE WATER | | | |
|---|---|---|--------------------------------|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | 5 | 4 | 20 |
| 72 | 67 | 67 | 20 |
| 103 | 93 | 91 | 20 |
| 147 | 130 | 129 | 19 |
| 210 | 195 | 201 | 5 |
| 300 | 280 | 290 | 0 |
| 428 | 413 | 410 | 0 |
| 612 | 575 | 600 | 0 |

Calculations Based on Nominal Concentrations

$LC_{50} = 188.69 \mu\text{g/L}$

95 percent confidence limits, 174.66 - 203.86 $\mu\text{g/L}$

$WER = 188.69 \mu\text{g/L} / 25.22 \mu\text{g/L} = 7.48$

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

$LC_{50} = 173.37 \mu\text{g/L}$

95 percent confidence limits, 159.24 - 188.75 $\mu\text{g/L}$

$WER = 173.37 \mu\text{g/L} / 23.77 \mu\text{g/L} = 7.29$

TABLE 5. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO SOFT RECONSTITUTED WATER

| Results of WER Determination for Cardinal Plant Test Dates: September 12 - 14, 1995 SOFT RECONSTITUTED WATER | | | |
|--|---|---|--------------------------------|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | < 3 | < 3 | 20 |
| 6.6 | 6 | 5 | 20 |
| 9 | 9 | 8 | 20 |
| 13 | 12 | 11 | 20 |
| 19 | 18 | 18 | 19 |
| 27 | 25 | 25 | 7 |
| 39 | 38 | 39 | 0 |
| 56 | 52 | 54 | 0 |

Calculations Based on Nominal Concentrations

LC₅₀ = 25.22 µg/L
 95 percent confidence limits, 23.18 - 27.45 µg/L

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

LC₅₀ = 23.77 µg/L
 95 percent confidence limits, 21.73 - 25.99 µg/L

TABLE 6. RESULTS OF *Daphnia magna* 48-HOUR ACUTE TOXICITY TEST USING HEXAVALENT CHROMIUM ADDED TO MODERATELY HARD RECONSTITUTED WATER

| Results of WER Determination for Cardinal Plant Test Dates: September 12 - 14, 1995 MODERATELY HARD RECONSTITUTED WATER | | | |
|---|---|---|--------------------------------|
| Nominal Hexavalent chromium Concentration (ug/L) | Day 0 Measured Hexavalent chromium Concentration (ug/L) | Day 2 Measured Hexavalent chromium Concentration (ug/L) | Survival No. Alive (out of 20) |
| Control | < 3 | < 3 | 20 |
| 25 | 21 | 17 | 20 |
| 36 | 32 | 34 | 19 |
| 51 | 46 | 45 | 13 |
| 74 | 68 | 70 | 1 |
| 105 | 94 | 94 | 0 |
| 150 | 136 | 145 | 0 |

Calculations Based on Nominal Concentrations

LC₅₀ = 54.17 µg/L
 95 percent confidence limits, 49.44 - 59.35 µg/L

Calculations Based on Measured Concentrations (avg of day 0 and day 2 values)

LC₅₀ = 49.06 µg/L
 95 percent confidence limits, 44.57 - 54.02 µg/L

TABLE 7. WER SUMMARY

| Species | Dilution Water | LC ₅₀ Values | | |
|------------------------------------|-------------------------------------|-------------------------|----------------------|---------------------------|
| | | May 9, 1995 Sample | July 17, 1995 Sample | September 11, 1995 Sample |
| <i>D. magna</i> (primary test) | Soft Reconstituted Water | 24.84 µg/L | < 13.5 µg/L | 23.77 µg/L |
| | Moderately Hard Reconstituted Water | 53.83 µg/L | 57.10 µg/L | 49.06 µg/L |
| | Site Water | 196.66 µg/L | > 326 µg/L | 173.37 µg/L |
| | WER | 7.92 | > 24.15 | 7.29 |
| Fathead Minnow (secondary test) | Moderately Hard Reconstituted Water | | 78.71 mg/L | |
| | Site Water | | 69.36 mg/L | |
| | WER | not tested | 0.88 | not tested |

Estimated Costs to Conduct a Water-effect Ratio Study

As with any site-specific study, many variables and considerations factor into the level of effort and costs needed to perform a water-effect ratio (WER) study.

Many of the WERs conducted over the last ten years, however have been of a similar design for which a cost can be estimated. A WER study, based on the following set of conditions, may range from \$25,000 to \$30,000.

- It is a Method 1 study that is it is for a stream site in the vicinity of a plume.
- Static or static-renewal acute tests are used to derive a WER.
- Three separate WERs are conducted using the primary species and one using the secondary species.
- The chemical of interest is a metal.

Additional costs can be expected if:

- The WER study is for a large river or water body with multiple discharges and dynamic mixing situations.
- Static-renewal chronic tests or flow-through tests are used to derive a WER.
- More than three WERs are conducted.
- The costs of analysis are more expensive than routine metal analyses.